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Actions of phencyclidine on the perfused rabbit ear

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Phencyclidine potentiated the responses of the rabbit perfused ear and its isolated central artery to noradrenaline, adrenaline and periarterial nerve stimulation. The potentiation of nerve stimulation was more pronounced than the potentiation of exogenous noradrenaline or adrenaline. Where the access of noradrenaline was restricted to one surface of the artery only, phencyclidine caused a much greater potentiation of extraluminal than of intraluminal noradrenaline. A biphasic response to tyramine was observed, the secondary prolonged vasoconstrictor phase being blocked by phencyclidine. It is postulated that phencyclidine inhibits the uptake of both noradrenaline and tyramine by the adrenergic axons in the blood vessel wall in a manner similar to that suggested for cocaine.

THE vasopressor response produced by the anaesthetic agent, phencyclidine, arises from both direct and indirect sympathomimetic effects (Ilett, Jarrott & others, 1966). Phencyclidine also potentiates the pressor effects of noradrenaline and adrenaline and transiently reduces the pressor response to tyramine. Ilett & others (1966) speculated that phencyclidine might potentiate the effects of exogenous noradrenaline and adrenaline by inhibiting their uptake into the adrenergic axon. The antagonism of the effect of tyramine could result from phencyclidine also blocking the uptake of tyramine. Since cocaine can block the pressor effects of phencyclidine, it is possible that cocaine and phencyclidine compete for the same uptake site. Some cocaine-like properties of phencyclidine have been reported by Chen, Ensor & Bohner (1965) and we now describe experiments on rabbit isolated ear and its isolated central artery which support the hypothesis that the action of phencyclidine is cocaine-like.

Experimental

METHODS

Lop-eared rabbits, 2–3 kg, were used. Three kinds of preparation were used: 1. The isolated perfused central artery of the rabbit ear cannulated only at the proximal end as described by De la Lande & Rand (1965), i.e. single cannulated artery. 2. The isolated perfused central artery of the rabbit ear cannulated at both the distal and proximal ends (De la Lande, Cannell & Waterson, 1966), i.e. double cannulated artery. 3. The central artery of the rabbit ear perfused *in situ* with vascular changes recorded as described by De la Lande & Rand (1965), i.e. isolated perfused rabbit ear.

The central artery was perfused with Krebs bicarbonate solution at 37° by a roller pump at constant rate of flow (6–8 ml/min). Constrictions were recorded as an increase in perfusion pressure using either a Condon manometer or a Statham P23AC pressure transducer.

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Drugs were injected into the perfusion fluid immediately proximal to the preparation (intraluminal injection) or were added to the perfusion fluid (intraluminal perfusion) or were added to the bath fluid surrounding the isolated artery (extraluminal addition). Periarterial nerve stimulation was effected by bipolar platinum electrodes as described by De la Lande & Rand (1965).

Drugs and solution. Drugs used were (−)-adrenaline acid tartrate, (−)-noradrenaline acid tartrate, cocaine hydrochloride, phencyclidine hydrochloride, tyramine hydrochloride. Doses of noradrenaline and adrenaline are expressed as dose of free base and doses of tyramine, cocaine and phencyclidine as dose of salt.

In experiments where noradrenaline was administered by intraluminal perfusion the Krebs perfusing fluid contained 200 μg/ml ascorbic acid.

Results

EFFECT OF PHENCYCLIDINE ON NORADRENALINE, ADRENALINE AND NERVE STIMULATION

Intraluminal injection of phencyclidine (0·5–1·5 mg) into the single cannulated artery preparation potentiated the intensity of the responses to periarterial nerve stimulation more than to intraluminal injections of noradrenaline and the duration of the potentiation was 30–40 min. The same

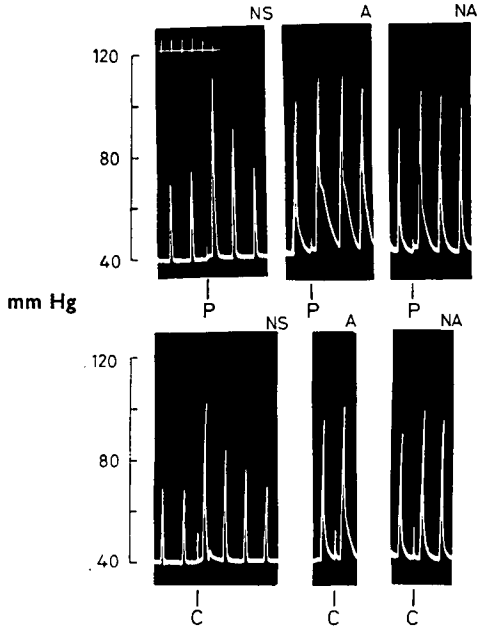

FIG. 1. Perfused rabbit ear. The effect of an injection of 25 μg of phencyclidine (P) and of 25 μg of cocaine (C) given 30 sec before nerve stimulation (NS, 40V, 1 msec, 20/sec for 10 sec); adrenaline (A, 5 ng); and noradrenaline (NA, 5 ng). Nerve stimulation is potentiated more than adrenaline or noradrenaline. Time in min.

effect could be produced by perfusing low concentrations of phencyclidine (5 μg/ml) through the artery for 15–20 min. The effect of phencyclidine was usually reversible 20–40 min after stopping the perfusion. In some experiments it was possible to find a concentration which would potentiate the responses to nerve stimulation without any effect on injected noradrenaline. Intraluminal perfusions of high concentrations of phencyclidine caused a reduction in the noradrenaline response whilst that to nerve stimulation was increased.

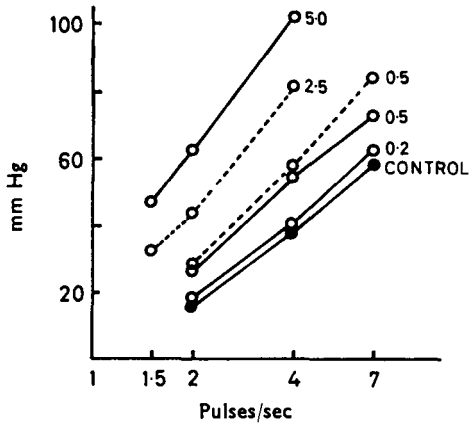

FIG. 2. Perfused rabbit ear. The effect of perfusions of phencyclidine ○——○ and cocaine ○ - - - ○ on the log frequency-response line to periarterial nerve stimulation (60 V, 0·05 msec, stimulated for 10 sec). Control responses are represented by ●——●. The doses of phencyclidine and cocaine are in μg/ml. Duration of perfusion was 14 min at flow rate of 8 ml/min.

A more marked potentiation of the intensity of the response to nerve stimulation than of that to an intraluminal injection of noradrenaline or adrenaline was also observed on the isolated perfused ear preparation. The effects of an intraluminal injection of 25 μg of phencyclidine and of 25 μg of cocaine are illustrated in Fig. 1.

Similar results were obtained if low concentrations of phencyclidine (0·5–5·0 μg/ml) were perfused through the ear for 14–20 min. The potentiating effect of phencyclidine on the responses to periarterial nerve stimulation increased with the concentration of phencyclidine and a parallel shift in the log frequency-response line occurred (Fig. 2). It was difficult to investigate the effects of larger concentrations of phencyclidine given by intraluminal perfusion since the resting perfusion pressure increased significantly and normal sensitivity to nerve stimulation and to noradrenaline did not readily return.

The relative potency of noradrenaline administered to the intraluminal and extraluminal surfaces of the double cannulated artery varied with the sensitivity of the preparation, but noradrenaline was always more active by intraluminal perfusion. When 10 μg/ml of phencyclidine was perfused intraluminally, the noradrenaline added extraluminally was potentiated much more than noradrenaline perfused intraluminally. The effect on

noradrenaline was easily reversed on reverting to normal Krebs solution (Fig. 3).

There was no increase in the response to single doses of noradrenaline administered by intraluminal injection. In some experiments continued perfusion of phencyclidine caused an increase in resting perfusion pressure.

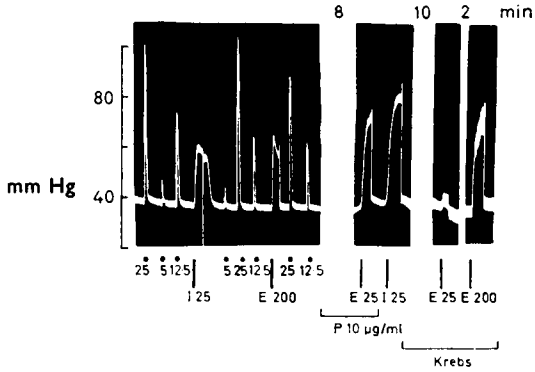

FIG. 3. Double cannulated artery. Unqualified figures refer to injections of noradrenaline (ng). An intraluminal perfusion of 25 ng/ml of noradrenaline (I25) gave the same response as 200 ng/ml bath fluid of noradrenaline added extraluminally (E25). After an intraluminal perfusion of 10 μg/ml of phencyclidine (P) for 15 min only 25 ng/ml of noradrenaline extraluminally (E25) was required to give a similar response to 25 ng/ml of noradrenaline intraluminally (I25). On reverting to normal Krebs solution the potentiation of extraluminal noradrenaline ceased.

EFFECT OF PHENCYCLIDINE ON TYRAMINE

The effect of phencyclidine on the response of the artery to tyramine was examined on the three kinds of preparation. The response to an intraluminal injection (10–30 μg) of tyramine was biphasic in both the

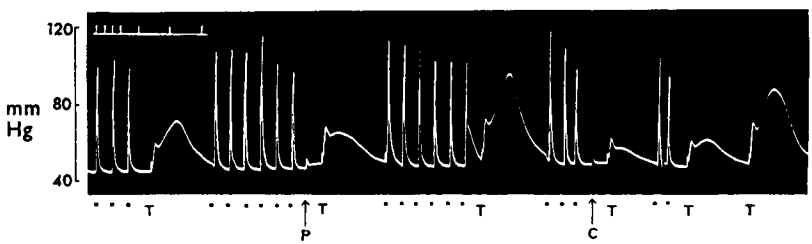

FIG. 4. Perfused rabbit ear. Unlabelled dots are injections of 10 ng noradrenaline. At T, 10 μg tyramine was injected. An injection of 25 μg phencyclidine (P) and of 25 μg cocaine (C) 30 sec before the tyramine reduced the secondary phase of the tyramine response. Time in min. During the tyramine response the drum speed was increased.

single cannulated artery and in the rabbit perfused ear preparation. There was an initial sharp vasoconstrictor response followed by a secondary more prolonged constriction. An intraluminal injection of either phencyclidine (25–50 μg) or cocaine (25–40 μg) 30 sec before the injection

of tyramine caused reduction or abolition of the secondary vasoconstriction but not the initial response (Fig. 4). The characteristic response to tyramine was restored as the phencyclidine or cocaine was washed out of the tissue by the perfusing Krebs solution. During most of these experiments injections of noradrenaline were made between doses of tyramine but the response to tyramine could be restored without the noradrenaline.

On the double cannulated artery, the response to an intraluminal injection of tyramine (30–60 μg) was a transient and reproducible constriction. Tyramine (2–3 μg/ml bath fluid) added to the extraluminal surface of the artery caused a more prolonged constriction which was not easily reproducible unless high doses of noradrenaline were added extraluminally to prevent tachyphylaxis. A perfusion of phencyclidine (10 μg/ml) for 20 min completely blocked the response to tyramine given extraluminally whereas that to intraluminal injection was unaffected.

Discussion

Uptake into storage sites in adrenergic nerves is now considered to play a major role in the termination of the response to noradrenaline released by nerve stimulation. Other sympathomimetic amines, including adrenaline, can also be taken up by adrenergic nerves (Iversen, 1965). Cocaine blocks this active uptake of noradrenaline (Whitby, Axelrod & Hertting, 1960; Iversen, 1965) and it has been suggested that this property of cocaine explains its potentiation of the effects of nerve stimulation and of noradrenaline *in vivo* (Trendelenburg, 1966). On the isolated ear central artery and the perfused whole ear of the rabbit, phencyclidine caused a greater potentiation of the effects of nerve stimulation than it did those of exogenous noradrenaline, an effect already reported for cocaine (De la Lande & others, 1966), and the degree of potentiation of nerve stimulation by phencyclidine and cocaine increased with increasing drug concentration.

On the double cannulated artery, an intraluminal perfusion of phencyclidine caused a marked potentiation of the effects of noradrenaline added to the extraluminal surface whilst having only a small effect on the intraluminal perfusion of noradrenaline. Since the adrenergic axons in the rabbit ear central artery are situated at the outer perimeter of the smooth muscle layer, the concentration of noradrenaline reaching the smooth muscle from the extraluminal surface will be reduced by uptake into the adrenergic nerve terminals (Gillespie, 1966; De la Lande & Waterson, 1967). The sensitizing action of phencyclidine is preferential for the extraluminal noradrenaline and can be explained if phencyclidine blocks the uptake mechanism of the adrenergic axon membrane, as has been described for cocaine on this preparation by De la Lande & Waterson (1967). Single intraluminal injections of noradrenaline on the double cannulated artery were not potentiated by phencyclidine although intraluminal injections into the single cannulated artery and the whole ear showed marked potentiation. If the differences between the three types

of preparation described are considered, this observation is consistent with the concept that the sensitizing action of phencyclidine is due to a block of uptake of noradrenaline acting from the extraluminal surface. In the single cannulated artery any drug injected intraluminally reaches the extraluminal surface, since the external bathing fluid is continuous with the perfusing fluid, and in the whole ear intraluminally injected drugs probably reach the extraluminal surface via the vascular system of the ear. If the artery is cannulated at both ends, noradrenaline injected intraluminally does not reach the extraluminal surface.

A biphasic response was obtained on injecting tyramine into the perfused rabbit ear or into the single cannulated artery. Phencyclidine blocked the secondary, more prolonged, phase of the vasoconstrictor response. Farmer (1966) observed a biphasic response of tyramine on the central artery and showed that the secondary phase was due to noradrenaline release since it was abolished by reserpine, sympathetic denervation or cocaine. Cocaine inhibits responses to tyramine by blocking uptake of tyramine into the adrenergic axon thus preventing release of endogenous noradrenaline (Furchgott, Kirpekar & others, 1963). Thus a block of the secondary phase of the tyramine response by phencyclidine would suggest that phencyclidine can block the uptake of tyramine. An intraluminal injection of tyramine caused what is possibly a direct sympathomimetic effect and an extraluminal injection the indirect effects (Waterson & De la Lande, personal communication). The observation that phencyclidine blocked only the extraluminal response to tyramine supports the concept that phencyclidine blocks the uptake of tyramine into the adrenergic nerve terminals. The similarity in the response to tyramine on the whole ear, and on the single cannulated preparation of the central artery, and also the responses observed when the artery is cannulated at both ends, can be explained by considering the surfaces of the artery to which the drug has access in the three kinds of preparation. In the first two preparations tyramine is in contact with both surfaces of the artery whereas in the double cannulated artery the drug has access only to the surface of the artery to which it is applied.

The rise in perfusion pressure observed after perfusions of phencyclidine or cocaine could be caused by indirect sympathomimetic effects of phencyclidine (Chen & others, 1965; Ilett & others, 1966) and cocaine (Teeters, Koppanyi & Cowan, 1963; Maengwyn-Davies & Koppanyi, 1966). Alternatively, the rise could be due to the drugs blocking the re-uptake of spontaneously released noradrenaline. Either mechanism would result in an increased concentration of noradrenaline in the region of the receptors.

Thus the observed effects of phencyclidine on the blood vessels of the rabbit ear can be ascribed to its ability to (*a*) block the uptake of exogenous noradrenaline, or noradrenaline released on nerve stimulation, into the adrenergic nerve terminals; (*b*) block the uptake of tyramine and thus prevent the release of noradrenaline by tyramine and (*c*) possibly release stored noradrenaline. In each of these respects its actions are similar to those previously reported by other workers for cocaine.

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